**Supplementary figure**

**AP3B1 facilitates PDIA3/ERP57 function to regulate rabies virus glycoprotein selective degradation and viral entry**

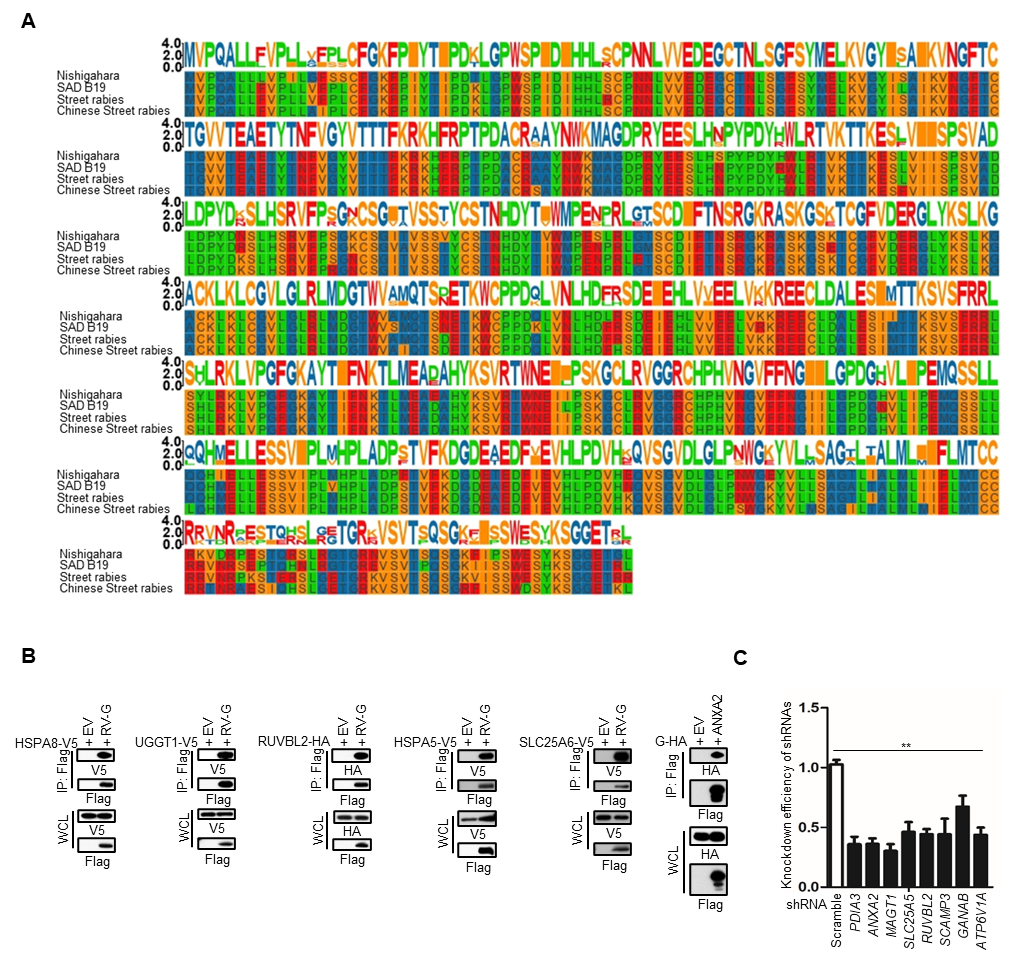
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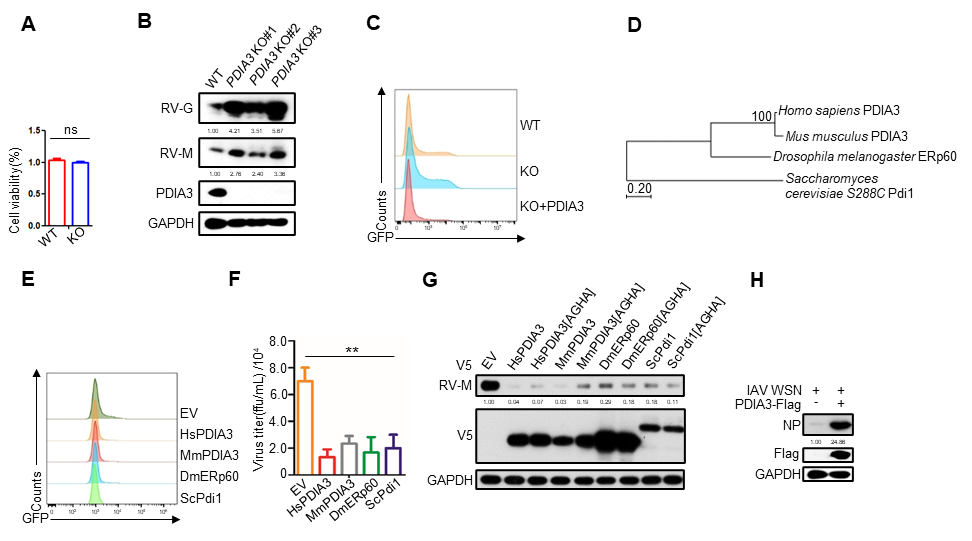
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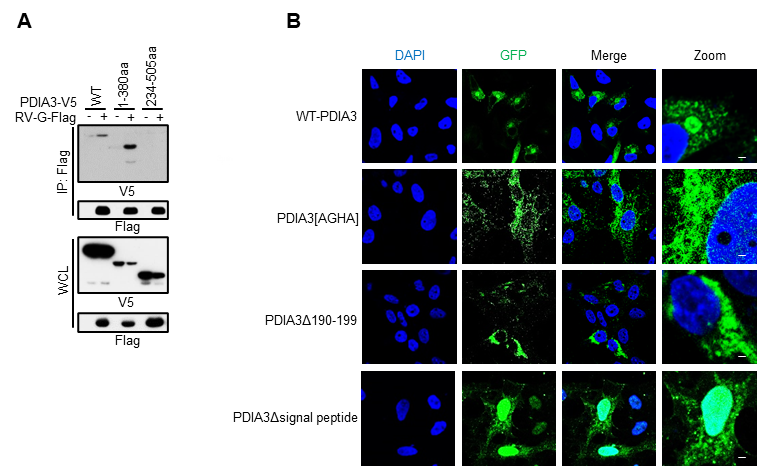
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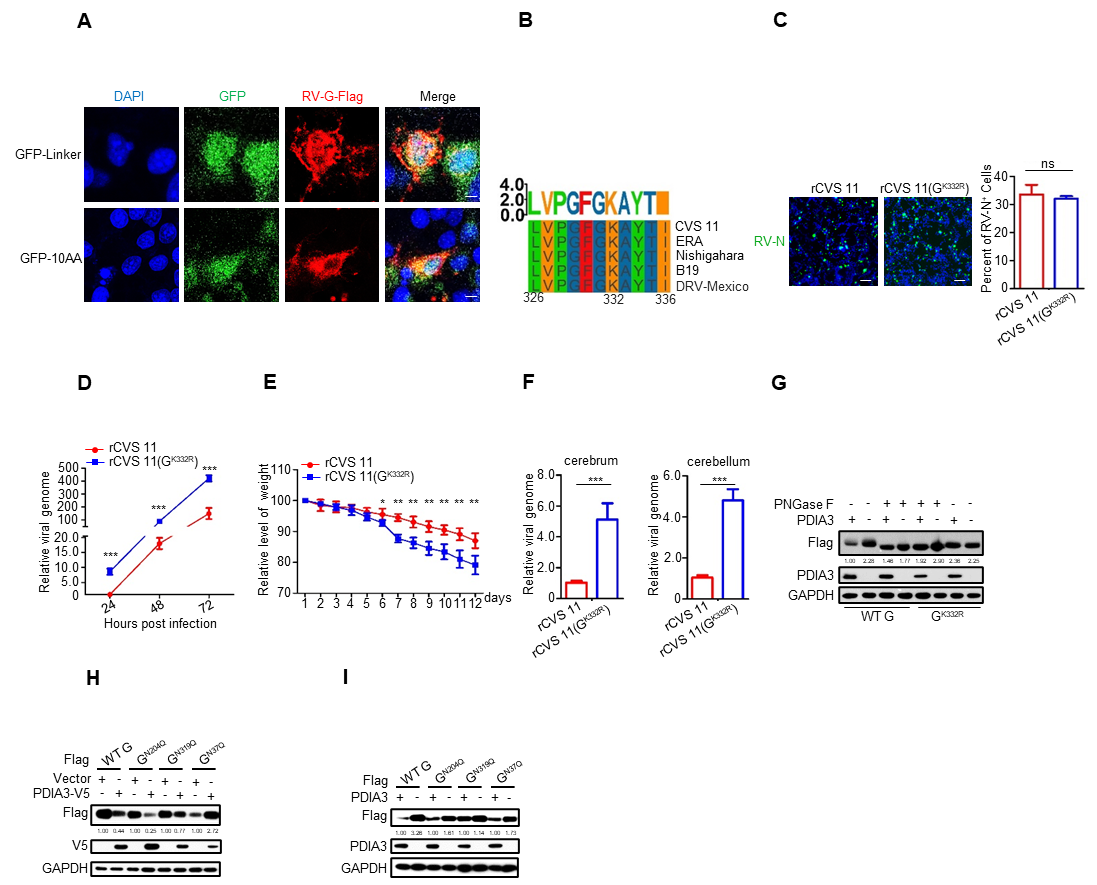
**Figure S1**. Identification of candidates for inhibiting RABV infection. (**A**) Ortholog alignment analysis of G proteins of Nishigahara, SAD B19, street rabies virus, and Chinese street rabies virus by Tbtool software. (**B**) Binding of RV-G to HSPA8, UGGT1, RUVBL2, HSPA5, SLC25A6 and ANXA2 were confirmed with co-immunoprecipitation. (**C**) qRT-PCR to validate the knockdown efficiency of part of shRNAs. n = 3 independent experiments; data are presented as mean ± SD. \*\**p <*0.01.



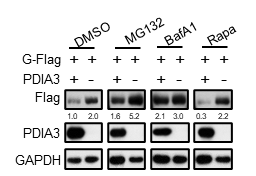
**Figure S2**. PDIA3 inhibits RABV infection. (**A**) Cell viability of HEK293 and *PDIA3* KO cells determined by MTT assays. n = 3 independent experiments; data are presented as mean ± SD. ns is not significant. (**B**) Western blot of RABV viral protein level at forty-eight h.p.i. HEK293 and three clones of *PDIA3* KO cells. Cells were infected with RABV strain CVS 11. (**C**) The percentage of GFP+ cells was determined via flow cytometry. HEK293, *PDIA3* KO and *PDIA3* KO overexpressing PDIA3-Flag tagged cell lines were treated with RABV-EGFP for forty-eight h. (**D**) Phylogenetic analysis of PDIA3/PDI from different species. (**E**) SH-SY5Y cells respectively transfected with EV, *Homo sapiens PDIA3* (*HsPDIA3*), *Mus musculus* Pdia3 (*MmPdia3*), *Drosophila melanogaster ERp60* (*DmERp60*), *Saccharomyces cerevisiae S288C PDI1* (*ScPDI1*), were treated with RABV-GFP for forty-eight h. Percent of GFP+ cells of these groups were quantified by flow cytometry. (**F**) SH-SY5Y cells respectively transfected with EV, *HsPDIA3*, *MmPdia3*, *DmERp60* and *ScPDI1* were treated with CVS 11 for forty-eight h. Supernatants were collected and virus titers were measured by a focus-forming assay. n = 3 independent experiments; data are presented as mean ± SD. \*\**p*< 0.01. (**G**) HEK293 cells were transfected with V5-tagged EV, *HsPDIA3*, *HsPDIA3[AGHA]*, *MmPdia3*, *MmPdia3[AGHA]*, *DmERp60*, *DmERp60[AGHA]*, *ScPDI1*, *ScPDI1[AGHA]*, respectively and treated with RABV for forty-eight h. RABV G protein levels were tested by western blot. (**H**) Western blot of IAV NP level at eighteen h.p.i. in HEK293 cells transfected with GFP or *HsPDIA3* fused with Flag tag. Cells were infected with IAV WSN.



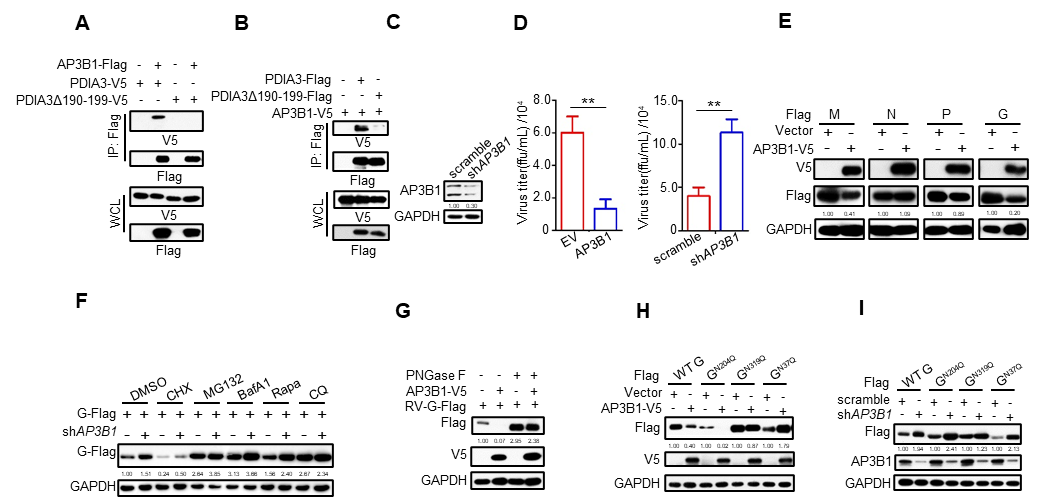
**Figure S3**. The 1-380aa of PDIA3 interacts with the RABV G protein. (**A**) Binding of PDIA3 truncations 1-380 aa, 234-505 aa fused-V5 to RABV G protein fused with Flag (RV-G-Flag), determined by Flag-IP and IB with anti-Flag and anti-V5. (**B**) Confocal analysis of EGFP-tagged WT PDIA3, PDIA3[AGHA], PDIA3Δ190-199, PDIA3Δsignal peptide in HeLa cells. Scale bar: 2 μm.



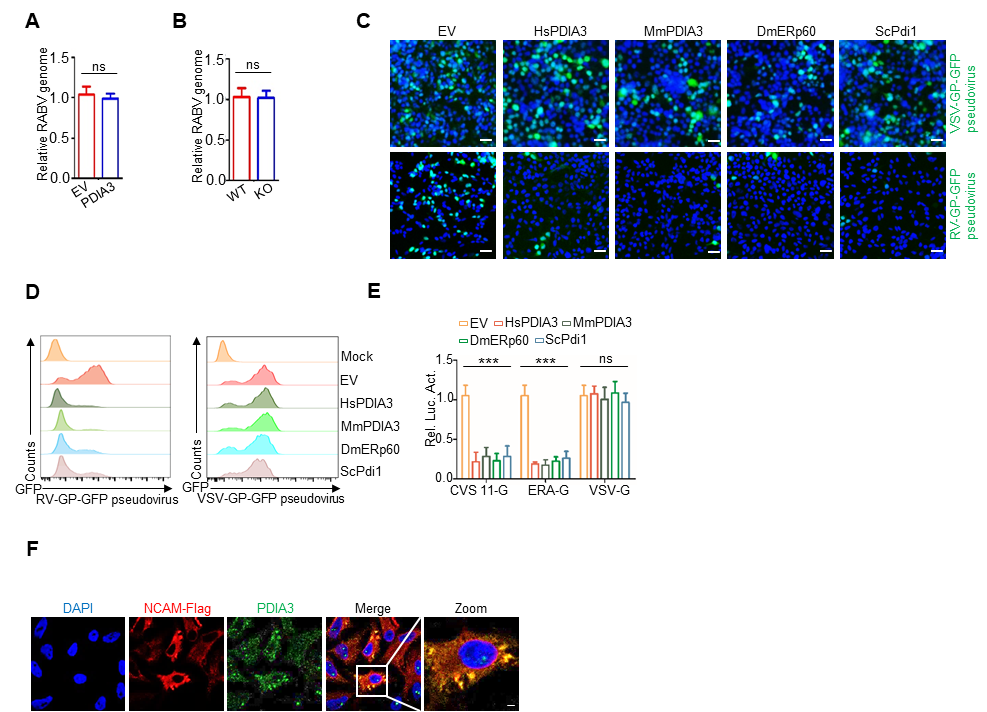
**Figure S4**. PDIA3 promotes the selective degradation of RABV G protein. (**A**) Confocal analysis of 190-199 aa fused to GFP and RV-G-Flag in A549 cells. Scale bar: 2 μm. (**B**) Ortholog alignment analysis of the residue 326-336 amino acids of glycoprotein from different RABV strains. (**C**) Images of rCVS 11 and rCVS 11(GK332R) infected cells at MOI of 3. Cells were fixed at twelve h post-infection and stained with anti-RABV N antibody for RABV N (green) and Hoechst 33342 for the nucleus (blue). Scale bar: 20 μm. The percent of RV-N+ was calculated by ImageJ software. n = 3 independent experiments; data are presented as mean ± SD. ns is not significant. (**D**) Viral growth curve. Supernatants were collected at the indicated time points, and virus titers were measured by a focus forming assay. n = 3 independent experiments; data are presented as mean ± SD. \*\*\**p*<0.001. (**E**) Weight of mice that were treated with rCVS 11 and rCVS 11(GK332R). C57BL/6J mice were infected by nasal inhalation of rCVS 11 and rCVS 11(GK332R). The mice were monitored daily for body weight. n = 4 independent experiments; data are presented as mean ± SD. \**p*<0.05, \*\**p*< 0.01. (**F**) qRT-PCR analysis of RABV genome mRNA level of cerebrum and cerebellum from rCVS 11 and rCVS 11(GK332R) treated mice at five days post-infection. data are presented as mean ± SD. \*\*\**p* < 0.001. (**G**) Western blot of RABV G protein (RV-G-Flag) level in HEK293 and *PDIA3* KO cells treated with PNGase F. (**H**) Western blot of RABV G level with the key glycosylation site Asn37, Asn204, or Asn319 mutations in HEK293 cells which transiently expressed PDIA3. (**I**) Western blot of RABV G level with the key glycosylation site Asn37, Asn204, or Asn319 mutations in HEK293 and *PDIA3* KO cells.

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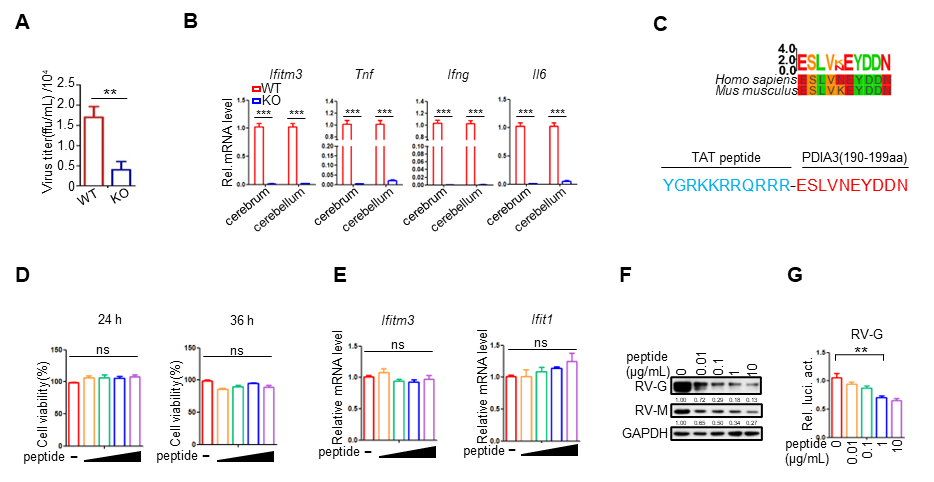
**Figure S5**. PDIA3 mediates the degradation of G protein via autophagosome-lysosome pathway. Immunoblot analysis of RABV CVS 11 G protein in HEK293 and *PDIA3* KO cells which were treated with MG132 (10 mM), BafA1(1 µM), and rapamycin (Rapa, 100 nM) for twelve h.



**Figure S6**. AP3B1 is essential for PDIA3 inhibiting rabies virus infection. (**A**) Binding of AP3B1 fused-Flag to PDIA3-V5 or PDIA3Δ190-199-V5, determined by Flag-IP and IB with anti-Flag and anti-V5. (**B**) Binding of PDIA3 or PDIA3Δ190-199 fused-Flag to AP3B1-V5, determined by Flag-IP and IB with anti-Flag and anti-V5. (**C**) Western blot to verify the knockdown efficiency of sh*AP3B1*. (**D**) HEK293 cells respectively transfected with EV, *AP3B1*, scramble shRNA and sh*AP3B1* were treated with CVS 11 for forty-eight h. Supernatants were collected and virus titers were measured by a focus forming assay. n = 3 independent experiments; data are presented as mean ± SD. \*\**p<*0.01. (**E**) Western blot of RABV viral proteins level in HEK293 which transfected with EV or *AP3B1*. (**F**) Western blot of RABV G protein level in *AP3B1*-knockdown HEK293 cells which were treated with CHX (25 ug/mL), MG132 (10 mM), BafA1 (1 µM), rapamycin (Rapa, 100 nM), or chloroquine (CQ, 20 μM) for twelve h. (**G**) Western blot of RABV G protein (RV-G-Flag) level in HEK293 cells which transfected with EV or *AP3B1* and treated with PNGase F. (**H**) Western blot of RABV G level with the key glycosylation site Asn37, Asn204, or Asn319 mutations in HEK293 cells which transiently expressed AP3B1. (**I**) Western blot of RABV G level with the key glycosylation site Asn37, Asn204, or Asn319 mutations in *AP3B1*-knockdown HEK293 cells.



**Figure S7**. PDIA3 restricts RABV entry into host cells. (**A**) RABV viral genome RNA of HEK293 cells overexpressing PDIA3. HEK293 cells were transfected with *PDIA3*. RABV viral genome RNA was transfected into cells by Lipofectamine 2000. forty-eight h after RNA transfection, the viral RNA level of cells was quantified by qRT-PCR. ns is not significant. (**B**) RABV viral genome RNA of HEK293 and *PDIA3* KO cells. RABV viral genome RNA was transfected into cells by Lipofectamine 2000. forty-eight h after RNA transfection, the viral RNA level of cells was quantified by qRT-PCR. ns is not significant. (**C**) IFA of CVS 11 and VSV glycoprotein-GFP pseudovirus in SH-SY5Y cells which transfected with EV, *HsPDIA3*, *MmPdia3*, *DmERp60* and *ScPDI1*, respectively. Scale bar: 50 μm. (**D**) The percentage of GFP+ cells was determined via flow cytometry. CVS 11 and VSV glycoprotein-GFP-pseudovirus in SH-SY5Y cells lines transfected with EV, *HsPDIA3*, *MmPdia3*, *DmERp60* and *ScPDI1*, respectively. (**E**) Luciferase reporter activity of CVS 11, ERA, and VSV glycoprotein pseudotyped viruses in HEK293 cells which transfected with EV, *HsPDIA3*, *MmPdia3*, *DmERp60* and *ScPDI1*, respectively. n = 3 independent experiments; data are presented as mean ± SD. \*\*\**p*< 0.001. ns is not significant. (**F**) Confocal analysis of NCAM-Flag with endogenous PDIA3 in HeLa cells. Scale bar: 2 μm.



**Figure S8**. The 190–199 amino-acid region of PDIA3 is sufficient to defend against RABV. (**A**) HEK293 and *PDIA3* KO cells were treated with CVS 11 for forty-eight h. Supernatants were collected and virus titers were measured by a focus forming assay. n = 3 independent experiments; data are presented as mean ± SD. \*\**p*< 0.01. (**B**) qRT-PCR analysis of *Ifitm3,* *Tnf, Ifng* and *Il6* mRNA level of cerebrum from mice whom administered with RABV from supernatants of HEK293 or *PDIA3* KO cells according to Figure 8B. n = 3 independent experiments; data are presented as mean ± SD. \*\*\**p*< 0.001. (**C**) Ortholog alignment analysis of the 190-199 aa residues of HsPDIA3 and MmPDIA3. Schematic diagram of TAT-PDIA3 peptide (190-199aa). (**D**) *In vitro* cytotoxicity of HEK293 cells treated with TAT-PDIA3 peptide in different concentrations, determined by MTT assays. n = 3 independent experiments; data are presented as mean ± SD. ns is not significant. (**E**) qRT-PCR analysis of *Ifitm3* and *Ifit1* mRNA levels in HEK293 cells with or without treating TAT-PDIA3 peptide in different concentrations for twenty-four h. n = 3 independent experiments; data are presented as mean ± SD. ns is not significant. (**F**) Western blot of RABV viral protein levels in HEK293 cells with or without treating TAT-PDIA3 peptide in different concentration. The cells were challenged with RABV CVS 11 for forty-eight h. (**G**) Luciferase reporter activity of RABV glycoprotein pseudotyped viruses (RV-G) in HEK293 cell with or without treating TAT-PDIA3 peptide in different concentration. n = 3 independent experiments; data are presented as mean ± SD. \*\**p<* 0.01.